HHS Public Access

Author manuscript

J Invest Dermatol. Author manuscript; available in PMC 2014 January 01.

Published in final edited form as:

J Invest Dermatol. 2013 July ; 133(7): 1742–1751. doi:10.1038/jid.2013.68.

TREM-1 as a Potential Therapeutic Target in Psoriasis

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Abstract

Our group recently described a population of antigen presenting cells that appear to be critical in psoriasis pathogenesis, termed inflammatory myeloid dendritic cells ($CD11c^+$ $BDCA1^-$). *Triggering receptor expressed on myeloid cells type-1 (TREM-1) Signaling* was a major canonical pathway in the published transcriptome of these cells. TREM-1 is a member of the immunoglobulin superfamily, active through the DAP12 signaling pathway, with an unknown ligand. Activation through TREM-1 induces inflammatory cytokines including IL-8, MCP/CCL2 and TNF. We now show that TREM-1 was expressed in the skin of healthy and psoriatic patients, and there was increased soluble TREM-1 in the circulation of psoriasis patients. In psoriasis lesions, TREM-1 was co-localized with dendritic cells as well as $CD31^+$ endothelial cells. TREM-1 expression was reduced with successful NB-UVB, etanercept and anti-IL-17 treatments. An *in vitro* model of PGN-activated monocytes as inflammatory myeloid DCs was developed to study TREM-1 blockade, and treatment with a TREM-1 blocking chimera decreased allogeneic Th17 activation as well as IL-17 production. Furthermore, TREM-1 blockade of *ex vivo* psoriatic dendritic cells in an alloMLR also showed a decrease in IL-17. Together, these data suggest that the TREM-1 signaling pathway may be a previously unidentified therapeutic target to prevent the effects of inflammatory myeloid DCs in psoriasis.

INTRODUCTION

Psoriasis is a common inflammatory skin disease of unknown etiology, and dendritic cells (DCs) are thought to play an important role in the pathogenesis of skin lesions (Lowes *et al.*, 2007). There are two main subgroups of myeloid DCs in psoriasis: blood dendritic cell

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CONFLICT OF INTEREST

The authors do not have any conflict of interest.

antigen (BDCA)-1/CD1c$^+$ resident DCs, and a subset termed “inflammatory DCs” which includes “TNF- and iNOS-producing DCs” or Tip-DCs (Zaba *et al.*, 2009b). These Tip-DCs were first found in a murine model of *Listeria monocytogenes* infection, and their development is dependent on CCR2 and MyD88 (Serbina *et al.*, 2003a; Serbina *et al.*, 2003b). Further studies have shown that human inflammatory DCs similarly produce TNF and iNOS (Lowes *et al.*, 2005), are negative for BDCA-1, and also produce IL-12p40, IL-23p19, and IL-20 (Wang *et al.*, 2006; Zaba *et al.*, 2009a; Zaba *et al.*, 2010).

To further characterize these inflammatory DCs, in 2010 we defined their transcriptome using global genomic profiling (Zaba *et al.*, 2010). FACS-sorted psoriasis inflammatory DCs (HLA-DR$^+$ CD11c$^+$BDCA-1$^-$ cells) and resident DCs (HLA-DR$^+$ CD11c$^+$BDCA-1$^+$ cells) were hybridized, and their expression profiles were compared to generate a list of differentially expressed genes (DEGs), defining the psoriatic inflammatory myeloid DCs transcriptome. Using this approach, TRAIL and TLR2 were identified as markers of inflammatory DCs (Zaba *et al.*, 2010). Now, while interrogating this transcriptome using bioinformatics approaches (Ingenuity Pathway Analysis), *Triggering receptor expressed on myeloid cells 1 (TREM-1) Signaling* was identified as the third highest canonical pathway enriched in this transcriptome, with *NK cell* and *IL17A* signaling being the top two pathways.

TREM-1 (CD354), first described over ten years ago by Bouchon *et al,* is a member of the immunoglobulin superfamily, constitutively expressed on monocytes and neutrophils in peripheral blood (Bouchon *et al.*, 2000; Bouchon *et al.*, 2001; Matesanz-Isabel *et al.*, 2011). It has also been identified on airway epithelial cells, hepatic endothelial cells, NK cells, DCs, B and T cells (Chen *et al.*, 2008; Matesanz-Isabel *et al.*, 2011; Rigo *et al.*, 2012). TREM-1 is activated synergistically with TLR agonists and interacts with epidermal antimicrobial peptide (cathelicidin/LL37) (Amatngalim *et al.*, 2011). As TREM-1 lacks an intracellular domain, downstream signaling by an unknown ligand is mediated through a DAP12 adaptor protein (Tessarz and Cerwenka, 2008). Several transcription factors are activated including JAK2, STAT3/5, and NFκB. Ultimately, activation of TREM-1 signaling pathway results in the downstream production of a variety of pro-inflammatory cytokines, including TNF, MCP/CCL2, MIP-1α and IL-8, amplifying innate and adaptive immune responses. However, soluble TREM-1 may play a role as an inhibitor by binding the unknown ligand and preventing binding to the TREM-1 surface receptor (Bouchon *et al.*, 2001).

TREM-1 may be involved in infectious and non-infectious inflammation, as well as autoimmunity. TREM-1 pathway activation has been reported in pneumonia, pancreatitis, peptic ulcer disease, sepsis, chronic obstructive pulmonary disease and gout (Ford and McVicar, 2009). In inflammatory bowel disease, soluble TREM-1 was elevated in serum and expressed on CD68$^+$ macrophages in the intestinal lamina propria (Park *et al.*, 2009; Schenk *et al.*, 2007). Furthermore, TREM-1 blockade has been shown to improve the clinical course of a murine model of inflammatory bowel disease (Schenk *et al.*, 2007), and prolong survival in murine sepsis (Gibot *et al.*, 2004). Recently, mice deficient in TREM-1 were generated, resulting in attenuated diethylnitrosamine induced hepatocellular carcinoma

carcinogenesis (Wu *et al.*, 2012), suggesting a role for TREM-1 in chronic inflammation associated with cancer progression.

Having identified *TREM-1 Signaling* in our transcriptome of psoriatic inflammatory myeloid DCs, we were interested in characterizing this pathway in psoriasis. TREM-1 was expressed on myeloid cells in the circulation of psoriatic patients as well as in lesions. Furthermore, TREM-1^+ cells were reduced in psoriatic lesions following successful treatment. TREM-1 blockade in an *in vitro* and *ex vivo* allogeneic MLR using two different types of activated antigen presenting cells (peptidoglycan (PGN)-activated monocytes and psoriatic lesional DCs) reduced IL-17 production, suggesting the functional significance of TREM-1 pathway in psoriasis.

RESULTS

TREM-1 Signaling pathway was identified in the transcriptome of psoriatic inflammatory DCs

Our group recently identified a population of $CD11c^+BDCA\text{-}1^-$ antigen presenting cells termed inflammatory myeloid dendritic cells in psoriasis (Zaba *et al.*, 2010). The published transcriptome of these FACS-sorted BDCA-1^+ versus BDCA-1^- DCs from psoriasis lesions was mined using Ingenuity Pathway Analysis (IPA) to identify biologically relevant pathways enriched in this transcriptome (Table S1 for the top 50 pathways with FDR<0.05). *TREM-1 Signaling* was the third highest canonical pathway in this analysis ($p=1.31\times10^{-7}$), behind *Natural Killer Cell Signaling* and *Role of IL17A in Psoriasis*. The canonical *TREM-1 Signaling* pathway is shown in Figure S1. The list of genes in this pathway that were identified in this transcriptome in inflammatory DCs and their fold change (FCH) are shown in Table S2.

In situ and circulating TREM-1 protein was increased in psoriasis

TREM-1 immunohistochemistry was performed in paired non-lesional (NL), lesional (LS) psoriasis and normal skin, and representative images are shown in Figure 1a and S2b (Sigma IgG2a clone) and Figure S2c (R&D Systems IgG1 clone). TREM-1 protein was present in the epidermis of all sections, and there were also scattered positive dermal cells. There was over a three-fold increase in TREM-1^+ cells in psoriasis lesions compared to NL tissues (n=10, p=0.002) (Figure 1b). Normal skin contained 299 $TREM^+$ cells/mm (n=3). Epidermal TREM-1 expression was confirmed by flow cytometry of keratinocytes from normal skin and psoriasis lesions using the R&D systems anti-TREM-1 clone (Ingersoll *et al.*, 2011) (n=3 for each condition) (Figure 1c).

The pattern of TREM-1 mRNA expression mirrored protein expression, with a six-fold increase in mRNA in LS skin compared to NL skin (n=10, p=0.005) (Figure 1d). Additionally, TREM-1 message was also detected through RNA-sequencing (RNAseq) of psoriasis NL vs LS skin in a pilot study (n=3) (Jabbari *et al.*, 2012). In that study, TREM-1 expression (RefSeq NM_178174) was increased FCH= 3.25 ($\log_2$FCH 1.7) with p=0.04 and FDR 0.15. There was less TREM-1 message in all three of the NL samples by RNAseq, compared to LS skin, although it did not reach statistical significance when corrected for

multiple hypothesis testing. Soluble TREM-1 was also increased in the serum of a group of moderate-to-severe psoriasis patients (n=7) compared to healthy volunteers (n=5) (mean soluble TREM-1 in healthy volunteers 153 pg/ml, in psoriasis patients 402 pg/ml, p=0.02) (Figure 1e).

Characterization of TREM-1$^+$ cells in psoriasis lesions

Two-color immunofluorescence was performed with TREM-1 and cellular markers CD11c, CD31, CD3, BDCA-1, CD163, and neutrophil elastase (n=3–5) (antibodies in Table S3). CD11c, which represents both resident and inflammatory myeloid DCs, co-expressed TREM-1 (Figure 2a). CD31$^+$ dermal endothelial cells also co-expressed abundant TREM-1 (Figure 2b). BDCA-1$^+$ DCs and occasional CD163$^+$ macrophages in psoriasis lesions co-expressed TREM-1 (Figure S3a, b). There were rare CD3$^+$ T cells that showed TREM-1 expression in light field IF microscopy, and confocal microscopy did not show any co-localization between CD3 and TREM-1 (Figure 2c). Neutrophils co-expressed TREM-1 (Figure S3c), as has been seen in other tissues and also in the circulation (Bouchon *et al.*, 2001). TREM-1 staining of BDCA1$^+$ resident and BDCA-1$^-$ inflammatory myeloid DCs in psoriasis was confirmed by FACS staining of dermal single cell suspensions from psoriasis shave biopsies (Figure S4).

TREM-1 expression was reduced with effective treatment

To further evaluate the potential role of TREM-1 in psoriasis, the change in protein expression, mRNA and the signaling pathway was studied in different clinical treatment trials (Johnson-Huang *et al.*, 2010; Krueger *et al.*, 2012; Zaba *et al.*, 2007; Zaba *et al.*, 2009c). We first studied a group of previously characterized patients with moderate-to-severe psoriasis who were treated with 6 weeks of narrow-band ultraviolet radiation (NB-UVB) (Johnson-Huang *et al.*, 2010). Tissue sections and RNA were available from patients who responded to this therapy (n=5) and those that did not (n=5), further defined in Supplemental Materials and Methods. Representative TREM-1 staining is shown for both a responder and non-responder (Figure 3a). The change in TREM-1$^+$ dermal cells in response to NB-UVB treatment was significantly different between responders and non-responders (p=0.05) (Figure 3b). In responders, a mean decrease of 75 TREM-1$^+$ cells was observed (p=0.3), whereas in non-responders, there was an increase of 163 TREM-1$^+$ cells (p=0.06). The TREM-1 mRNA response to NB-UVB treatment was also significantly different between responders and non-responders (p=0.05) (Figure 3c). In responders, a 16 fold reduction in TREM-1 mRNA was observed (p=0.04), whereas in non-responders, there was minimal change in TREM-1 mRNA (p=0.6).

In a second group of seven patients receiving the same NB-UVB treatment protocol for 12 weeks (patients described in Table S4), there was less soluble TREM-1 at 12 weeks (mean soluble TREM-1 at baseline 402 pg/ml and after treatment 303 pg/ml) (Figure 3d). When change in soluble TREM-1 was assessed with patients grouped as responders or non-responders by *PASI75* response (Table S4), there was a 1.8 fold decrease in responders (p=0.096), and no difference in the non-responders (p=0.97). Although the change in TREM-1 was not significant with treatment for all patients grouped as responders/non-

responders, a large Cohen's effect size (mean/SD) of 1.2 (r=0.51) was observed, indicating that lack of significance was most likely due to the small sample size.

In a published study of patients with moderate-to-severe psoriasis who were treated with TNF-blockade (etanercept) for 12 weeks (Zaba *et al.*, 2007; Zaba *et al.*, 2009c), the response profile of genes in the *TREM-1 Signaling* pathway was evaluated in the transcriptome of patients during treatment, compared to NL levels (Figure 3e). The *TREM-1 Signaling* pathway gene set was completely resolved in those who responded to etanercept treatment, while it did not return to baseline in non-responders.

We have introduced the concept of "residual disease genomic profile (RDGP)" to represent the remaining genomic expression in lesions at the end of successful treatment, i.e. genes that improve by less than 75% (Suárez-Fariñas *et al.*, 2010). In a recent study of psoriasis patients with ixekizumab, an anti-IL-17 treatment (Krueger *et al.*, 2012), 62% of the nine genes in the *TREM-1 Signaling* pathway (Figure S1 and Table S2) that were differentially regulated in psoriasis, improved by over 75% by 2 weeks (Figure 3f), compared to 69% of all psoriasis genes. For comparison with etanercept at the same time-point in treatment, 34% of TREM-1 genes had recovered compared to only 31% of all psoriasis genes. There was a 2.69 FCH average expression of these TREM-1 genes towards recovery with ixekizumab at 2 weeks, compared to 1.56 FCH for etanercept, and no change (1.07 FCH) for placebo (Figure 3g). Overall, these results support the potential importance of the TREM-1 signaling pathway being involved in psoriasis, and improvement in these genes is required for lesion improvement.

Establishing a model of PGN-treated monocytes to test TREM-1 as a therapeutic target in vitro

As mentioned above, *TREM-1 Signaling* was detected in the transcriptomes of inflammatory DCs. It was also evident in a recent meta-analysis of full thickness biopsies from almost 180 psoriatic patients (paired LS vs. NL) ($p=0.028$) (Tian *et al.*, 2012) (Figure 4a). The *TREM-1 Signaling* pathway was also elevated in keratinocytes transfected with mutations in CARD14 ($p=2.7\times10^{-10}$), a recently discovered cause of psoriasis (Jordan *et al.*, 2012a; Jordan *et al.*, 2012b), and in acute UVB treatment of full thickness skin of healthy volunteers ($p=2.9\times10^{-9}$) (Figure 4a) (Kennedy-Crispin *et al.*, 2012). For comparison, *IL-17 Signaling* and *NFκB Signaling* pathways are shown for these transcriptomes (Figure 4a).

In order to test the effects of TREM-1 blockade, an *in vitro* model with activation of the *TREM-1 Signaling* pathway was developed. Monocytes were chosen as they have a high level of TREM-1 expression in healthy volunteers (n=3) (Bouchon *et al.*, 2000; Matesanz-Isabel *et al.*, 2011) and in psoriasis patients (n=3) (Figure S5). Monocytes were treated overnight with Peptidoglycan (PGN) and *Heat Killed Listeria Monocytogenes* (HKLM), agonists of TLR2, a receptor that is abundant on inflammatory myeloid DCs (Zaba *et al.*, 2010). These TLR2-activated monocytes produced IL-23p40, IL-1β, IL-6, and TNF (n=3, Figure S6a); some IL-23 mRNA (n=3, Figure S6b); induced robust T cell proliferation (n=3, data not shown), and most importantly Th17/Th1 cells in an alloMLR (n=8, Figure S7). Additionally, monocytes were treated with PGN or HKLM and their genomic profiles compared to paired untreated monocytes. DEGs for each comparison were those with

FCH>1.5, FDR<0.01 (Table S5 and S6). A Venn diagram with differentially up- and down-regulated probe-sets is shown in Figure S8. Interrogating the transcriptomes of PGN- and HKLM-treated monocytes identified significant activation of the *TREM-1 Signaling* pathway (Figure 4a) ($p=2.7\times10^{-4}$ and 4.5×10^{-8}, respectively). As PGN is likely to be a more general agonist, we chose to use PGN-treated monocytes to continue these studies, considering that they presented a useful model to study TREM-1 blockade.

TREM-1 blockade reduced Th17 cells in an in vitro alloMLR

$CD14^+$ selected monocytes were cultured overnight with PGN, then washed, and co-cultured with allogeneic T cells in the presence of an inhibiting TREM-1 chimera (Bouchon *et al.*, 2000; Bouchon *et al.*, 2001), a construct with TREM-1 protein fused to Fc region of IgG1, added on day 1 and 3. T cells were harvested on day 7 and analyzed by intracellular cytokine staining (PMA and ionomycin activated cells), and cytokines measured in the supernatant. There was an approximately 50% reduction in the frequency of Th17 cells in the presence of TREM-1-IgG chimera compared to the IgG1 control antibody (n=2) (Figure 4b). In a second set of samples (n=4) there was significantly less IL-17 in the supernatant at day 7 (p=0.03), while there was no apparent reduction in IFNγ protein (Figure 4c).

TREM-1 blockade decreases Th17 cells in an ex vivo alloMLR

To pursue this observation further in an *ex vivo* model, dermal single cell suspensions from psoriatic shave biopsies (which contain predominantly DCs) were co-cultured with allogeneic T cells in the presence of TREM-1 blockade with the chimera (n=3). There was approximately a 50% reduction in IL-17 producing cells in the presence of TREM-1 blockade (representative image in Figure 5a). The percentage of $IFN\gamma^+IL\text{-}17^+$, $IFN\gamma^-IL\text{-}17^+$, total $IL\text{-}17^+$, and total $IFN\gamma^+$ cells showed that there was a decrease in IL-17-producing cells, but minimal reduction in IFNγ producing cells (Figure 5b). There was also a reduction in IL-17 production in the presence of the blocking TREM-1 chimera in this assay (Figure 5c). This response was of similar magnitude to the reduction in a parallel assay using ustekinumab (anti-p40), an FDA-approved biologic therapy for psoriasis (n=3; Figure 5d). Specifically, the mean reduction in total IL-17-producing cells was 66% with ustekinumab, comparable to the 50% reduction in IL-17 producing cells in the presence of TREM-1 blockade. These results indicate that TREM-1 blockade could reduce the effect of psoriatic DC activation of Th17 cells.

DISCUSSION

Psoriasis is a complex inflammatory process, and although much more is known about the involvement of the adaptive immune system and the central role of the IL-23-Th17 axis in this disease, questions still remain. These data show the presence of TREM-1 expression in psoriasis and a reduction with treatment, providing preliminary pre-clinical support for TREM-1 as a potential therapeutic target.

Initial review of this *TREM-1 Signaling* pathway suggests that this is a pathway involved in the innate immune system. Activation of the TREM-1 receptor (by its unknown ligand) augmented by TLR agonists, leads to DAP12 recruitment, and ultimately production of

cytokines such as IL-8, MCP/CCL2, and TNF. These mediators all contribute to acute inflammation, leading to recruitment of neutrophils and mononuclear cells. The high level of TREM-1 expression on circulating neutrophils in both normal and psoriatic subjects (Bouchon *et al.*, 2001), and recent evidence that neutrophils may produce IL-17 (Lin *et al.*, 2011) suggests a role in early inflammatory processes. The observation that acute high dosage NB-UVB also causes activation of the *TREM-1 Signaling* pathway likewise supports a role for TREM-1 in acute inflammation.

However, while TREM-1 signaling may be an early event in psoriasis pathogenesis, it may also be involved in ongoing inflammation. Early studies showed that TREM-1-activated monocytes could participate in both the early innate and adaptive immune responses (Bleharski *et al.*, 2003). In this setting, activation of TREM-1 on monocytes induced IL-8 and IL-1β, and also upregulated MHC Class II and co-stimulatory molecules such as CD86, leading to augmented T cell proliferation and IFNγ production. Furthermore, although innate early response cytokines such as IL-8 and IL-1β are elevated in psoriasis, they return to baseline in both those that respond to successful treatment with etanercept, and those that do not respond (Zaba *et al.*, 2009c). Therefore, it is of interest that TREM-1 signaling genes that were differentially regulated in psoriasis only returned to baseline in those that responded to treatment, but not in those patients that did not improve. TREM-1^+ cells and mRNA were also reduced only in responders to NB-UVB. IL-17 blockade also led to a reduction of TREM-1 genes as early as 2 weeks. These data suggest that there is persistent activation of the TREM-1 pathway during the maintenance of psoriasis lesions. In fact, the failure to turn off the TREM-1 signaling pathway may be part of the “psoriatic tendency” leading to maintenance of psoriasis lesions.

Recently, mutations in CARD14 were found in familial psoriasis vulgaris (Jordan *et al.*, 2012a; Jordan *et al.*, 2012b). Transfection of the CARD14 mutation into keratinocytes leads to increased NFκB activation, and production of many cytokines such as IL-8 and CCL20. For the psoriasis patients harboring mutations in CARD14, this suggests there may be a lower threshold for epidermal activation initiating psoriasis lesions. It was of interest then that TREM-1 protein expression was detected on keratinocytes by immunohistochemistry and FACS. Furthermore, the TREM-1 signaling pathway was also significant in the transcriptome of the keratinocytes with this CARD14 mutation. The relationship between TREM-1 and CARD14 remains to be evaluated, but they may share common mechanism of action as they can both activate NFκB.

Antimicrobial peptide human β-defensin 3 (BD3) upregulated co-stimulatory molecules CD80, CD86, and CD40 on monocytes via TLR1 and TLR2 in a MyD88-dependent manner (Funderburg *et al.*, 2007). This was specific to the myeloid cells, as BD3 did not have this effect on plasmacytoid DCs. These observations brought our attention to the concept that an endogenous keratinocyte peptide could activate monocytes to become potential antigen-presenting cells. Monocytes as precursors of inflammatory DCs have also been shown recently in a murine model of LPS-stimulation (Cheong *et al.*, 2010), as well as the initial description of Tip-DCs (Serbina *et al.*, 2003a; Serbina *et al.*, 2003b). Use of PGN-activated monocytes as a model of inflammatory DCs established a useful system to test TREM-1 blockade. Monocytes cultured overnight with PGN developed into antigen presenting cells

capable of robust allogeneic T cell proliferation, cytokine production, and Th1/Th17 activation. In this *in vitro* system, treatment of the PGN-activated monocytes with TREM-1 inhibiting chimera reduced the percentage of Th17 cells by half, and decreased the secretion of IL-17 into the supernatant, suggesting that blocking TREM-1 suppresses Th17 cells.

We have previously shown that both $CD11c^{+}BDCA\text{-}1^{+}$ resident DCs and $CD11c^{+}BDCA\text{-}1^{-}$ inflammatory myeloid DCs can activate allogeneic Th1/Th17 cells *ex vivo* (Zaba *et al.*, 2009a). While we first identified the TREM-1 Signaling pathway in the transcriptome of the inflammatory myeloid DCs, TREM-1 was present on both DC subsets, and the TREM-1 chimera decreased bulk psoriatic DCs induction of allogeneic Th17 cell activation and IL-17 production. This suggests that blockade of TREM-1 on both resident and inflammatory myeloid DCs may be a potential therapeutic strategy, as shown in Figure S9. Further studies are warranted to determine which population may be more important. As the mechanism of action of the TREM-1 chimera is considered to be via binding to the TREM-1 ligand, it is possible that DCs and/or T cells in the alloMLR may produce the ligand for TREM-1.

The addition of TREM-1 blockade in this *ex vivo* system reduced the percent Th17 cells and IL-17 production in a magnitude similar to IL-23p40 blockade (ustekinumab, Stelara, Janssen, an FDA-approved treatment for psoriasis). While time consuming and technically difficult due to the small numbers of cells available for study, this assay offers a useful method for assessing the effect of potential therapeutics in a human system to develop pre-clinical data. Overall, these data support the potential importance of TREM-1 in psoriatic inflammation and warrant further study of TREM-1 as a therapeutic target.

MATERIALS AND METHODS

Further details are available in Supplemental Material and Methods.

Skin and Blood Samples

Skin biopsies and blood were obtained from normal volunteers and psoriasis patients under a Rockefeller University Institutional Review Board approved protocol. Written informed consent was obtained and the study was performed in adherence with the Declaration of Helsinki Principles. De-identified normal skin was obtained from plastic surgeons. Skin biopsies for TREM-1 cell counting and PCR were from a previously published clinical treatment study with NB-UVB (Johnson-Huang *et al.*, 2010). Soluble TREM-1 was measured in a group of moderate-to-severe psoriasis patients receiving standard therapeutic NB-UVB 3 times a week for 12 weeks in a Rockefeller University Institutional Review Board approved protocol (clinicaltrials.gov NCT00844363). Characteristics of this group of patients are in Table S4 (three females, four males, mean age 47 years). The mean PASI at baseline was 22, mean PASI at 12 weeks treatment was 7, and mean reduction in PASI was 69%. 3/7 patients reached *PASI*75, and 6/7 reached *PASI50*.

Immunohistochemistry and Immunofluorescence

Immunohistochemistry was performed as previously described (Fuentes-Duculan *et al.*, 2010), with antibodies listed in Table S3a. Immunofluorescence was performed in lesional

psoriatic skin samples (n=3–5) as previously described (Fuentes-Duculan *et al.*, 2010), using antibodies and fluorochromes as outlined in Table S3a.

FACS

Keratinocytes were obtained from both psoriasis and normal skin after incubation with dispase, then trypsinization (0.25% tryspin, Gibco, Grand Island, NY). Keratinocytes were c-kit^{-}, HLA-DR^{-}. Dermal single cell suspensions were obtained from psoriasis shave biopsies as previously described (Zaba *et al.*, 2009a). PBMCs from healthy volunteers (n=5) and psoriasis patients (n=3) were obtained by Ficoll (GE Healthcare, Uppsala, Sweden) density centrifugation. FACS staining was performed as previously described (Zaba *et al.*, 2009a) (Table S3b).

TREM-1 Immunoassay

Soluble serum TREM-1 was measured by (TREM-1 DuoSet, R&D Systems, Minneapolis, MN) according to the manufacturers directions, in a group of patients before and after NB-UVB treatment (n=7) (Table S4), and compared to a group of healthy volunteers (n=5) (two females and 3 males, mean age 39 years).

RT-PCR

Quantitative RT-PCR was performed on samples using RNA from NB-UVB study as previously described (Johnson-Huang *et al.*, 2010) using TREM-1 primers (Hs_00218624_m1; Applied Biosystems, Carlsbad, CA), normalized to human acidic ribosomal protein gene (hARP) housekeeping gene (Johnson-Huang *et al.*, 2010).

Establishing an *in vitro* model for inflammatory DCs

Monocytes were treated with HKLM (10^8 cells/ml InvivoGen, San Diego, CA), PGN (5μg/ml, InvivoGen), or media, and RNA hybridized to HGU133A2.0 Affymetrix gene chips, as previously described (Zaba *et al.*, 2010). The list of DEGs for the treated monocytes analyzed in IPA was for FCH >1.5 and False Discovery Rate (FDR) <0.01. The data discussed in this publication have been deposited in the National Center for Biotechnology Information's Gene Expression Omnibus (GSE42305).

Allogeneic MLR with TREM-1 blockade

TREM-1 chimera (10μg/ml, R&D, 1278TR) or IgG1 (R&D) was added to the PGN-treated monocytes (alloMLR), or psoriasis dermal single cell suspensions (*ex vivo* alloMLR, i.e. unmanipulated dermal emigres from psoriatic skin) and bulk T cell (Rosette sep, Stem Cell Technologies) (1:10) co-culture at baseline and day 3. T cells were harvested on day 7–9 for intracellular cytokine staining, as previously described (Zaba *et al.*, 2009a). T cells with TREM-1 antibody at the same concentration, and low dose IL-2 to keep cells alive (20 IU/ml on days 1 and 3, Ebioscience) showed minimal Th17 activation (data not shown). IL-17 was measured in the supernatant harvested on the final day by ECL technique (IL-17) (Meso Scale Discovery, Gaithersburg, MD) or luminex (IFNγ) platforms, according to the manufacturers directions.

Statistics

LS versus NL cell counts and log transformed RT-PCR data were analyzed by paired t-tests. Soluble TREM-1 in serum of psoriasis patients was compared to healthy volunteers by Mann Whitney test. Analysis of cell counts, mRNA, soluble TREM-1 response to NB-UVB treatment was modeled using repeated measure ANOVA analysis, with between factor Response (Responders/Non-Responders) and within-subject factor Time (Pre/Post). Production of cytokines or mRNA, or percent Th17 cells by HKLM or PGN, was analyzed by repeated measures ANOVA. Effect of TREM-1 blockade on cytokine production in the supernatant was compared to isotype control by Mann Whitney test. To compare the proportion of genes in the TREM-1 pathway that improve after treatment, a McNeamar test of dependent proportions was used. Significance was accepted as $p<0.05$.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

ACKNOWLEGEMENTS

Research supported by National Institutes of Health (NIH) grant UL1 RR024143 from the National Center for Research Resources (NCRR). MAL, LH and JLH are supported by NIH 1R01AR060222; LMJ-H is supported by the Linda and Leonard Berkowitz Postdoctoral Fellowship. NG is supported by NIH MSTP grant GM07739. TL is supported by a National Psoriasis Foundation Discovery grant. We would like to thank Dr James Krueger for helpful discussions; Hanna Ning and Dr Hiroshi Mitsui for technical advice, and Drs Shivaprasad Bhuvanendran and Alison North from the Bio-Imaging Resource Center for technical support.

Abbreviations

TREM	triggering receptor expressed on myeloid cells
NB-UVB	narrow band ultra violet B
DC	dendritic cell
PASI	Psoriasis Area Severity Index
LS	lesional
NL	non-lesional
PGN	peptidoglycan
HKLM	heat killed listeria monocytogenes
DEG	differentially expressed genes
FCH	fold change
FDR	false discovery rate
hARP	human acidic ribosomal protein
IPA	Ingenuity Pathway Analysis
MLR	Mixed Leukocyte Reaction
BDCA	Blood dendritic cell antigen

RDGP residual disease genomic profile

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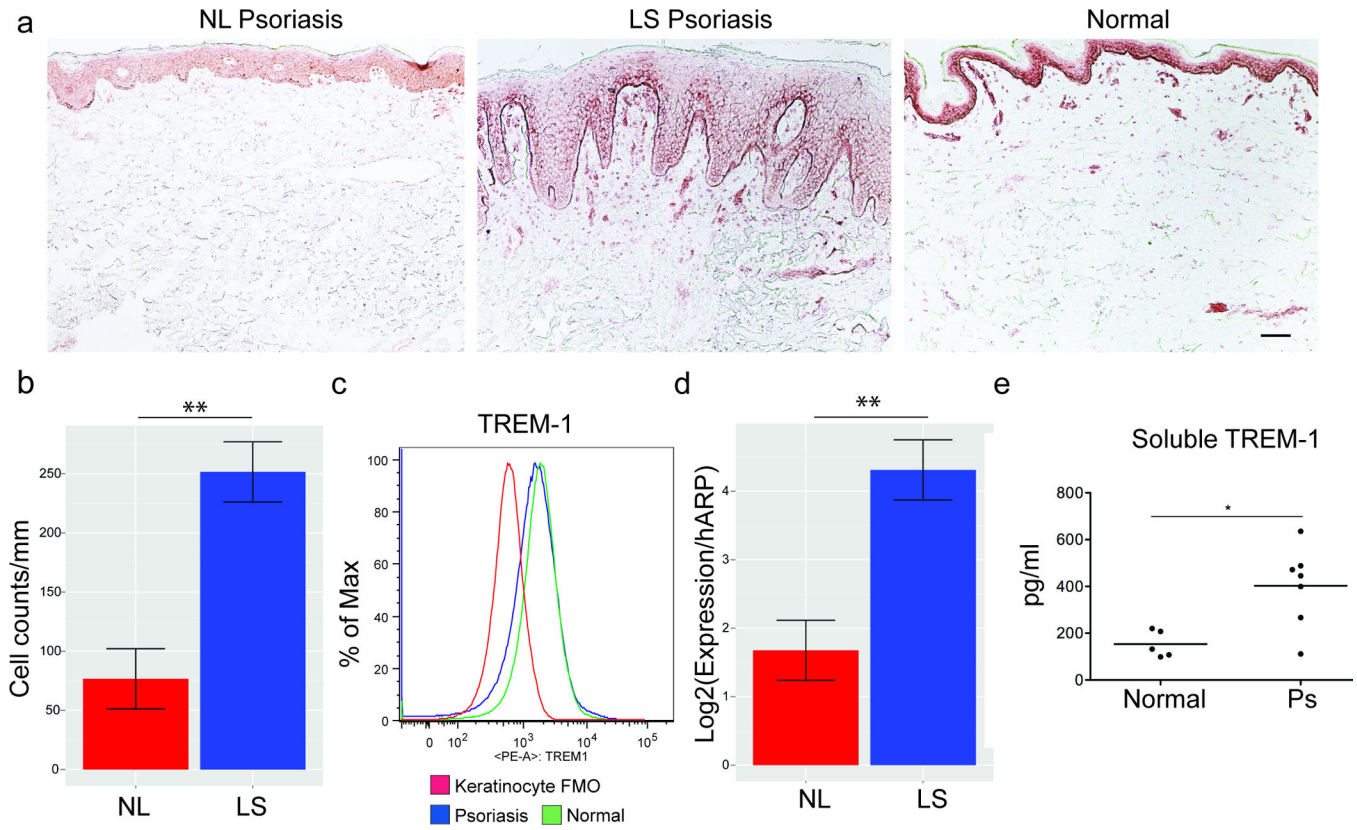

Figure 1. ***In situ*** **and circulating TREM-1 protein was increased in psoriasis**

(a) Representative TREM-1 protein expression in non-lesional (NL), lesional (LS) psoriasis skin and normal skin, with epidermal staining and TREM-1^{+} dermal cells, especially in lesional skin (Sigma IgG2a clone). (b) Dermal TREM^{+} cell counts in NL and LS skin (n=10). (c) TREM-1 expression on psoriatic (blue) and normal (green) keratinocytes by FACS (R&D Systems IgG1 clone; representative of n=3 for each group). Red line is negative control (FMO). (d) normalized TREM-1 mRNA expression in NL and LS skin (n=10). (e) Soluble TREM-1 in serum of healthy volunteers versus patients with psoriasis. *p<0.05, **p<0.01. Size bar is 100μm.

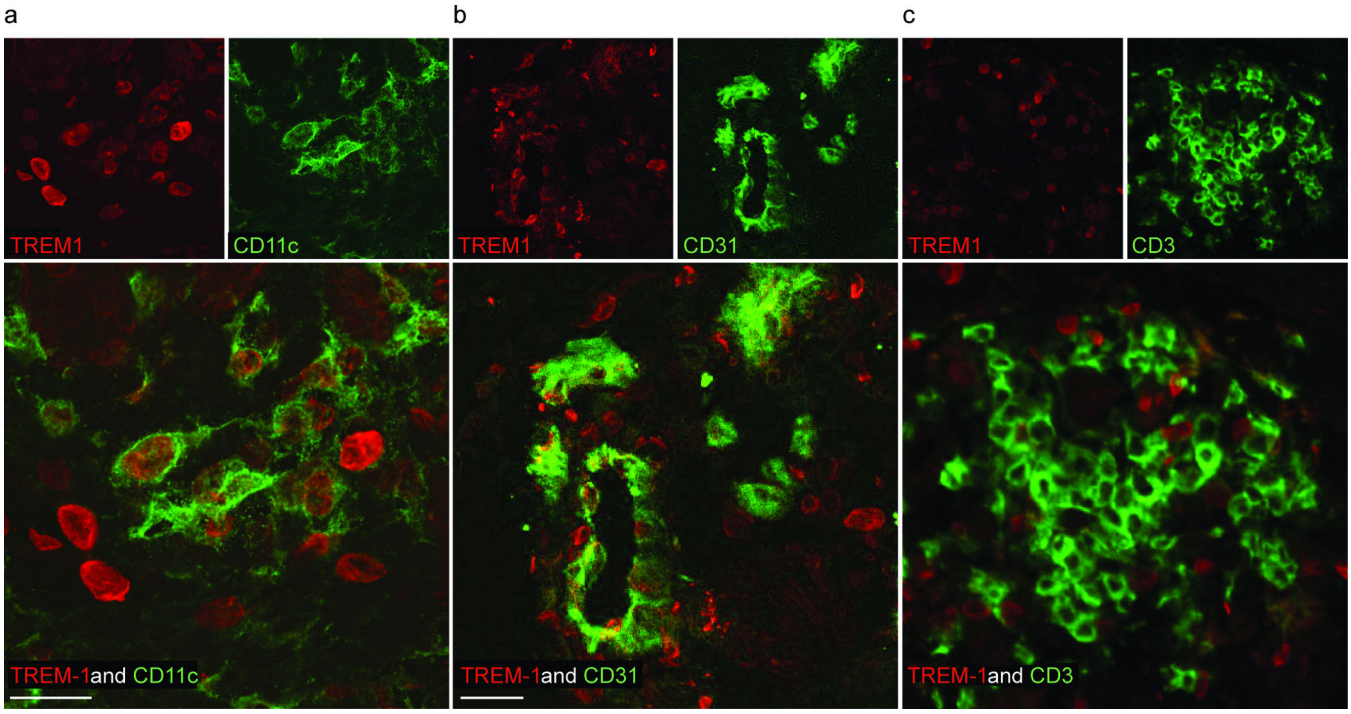

Figure 2. Characterization of TREM-1$^+$ cells in psoriasis lesions

Two color immunofluorescence of TREM-1 (red) with (a) CD11c$^+$ dendritic cells, (b) CD31$^+$ endothelial cells, and (c) CD3$^+$ T cells (each green). Size bar is 20μm.

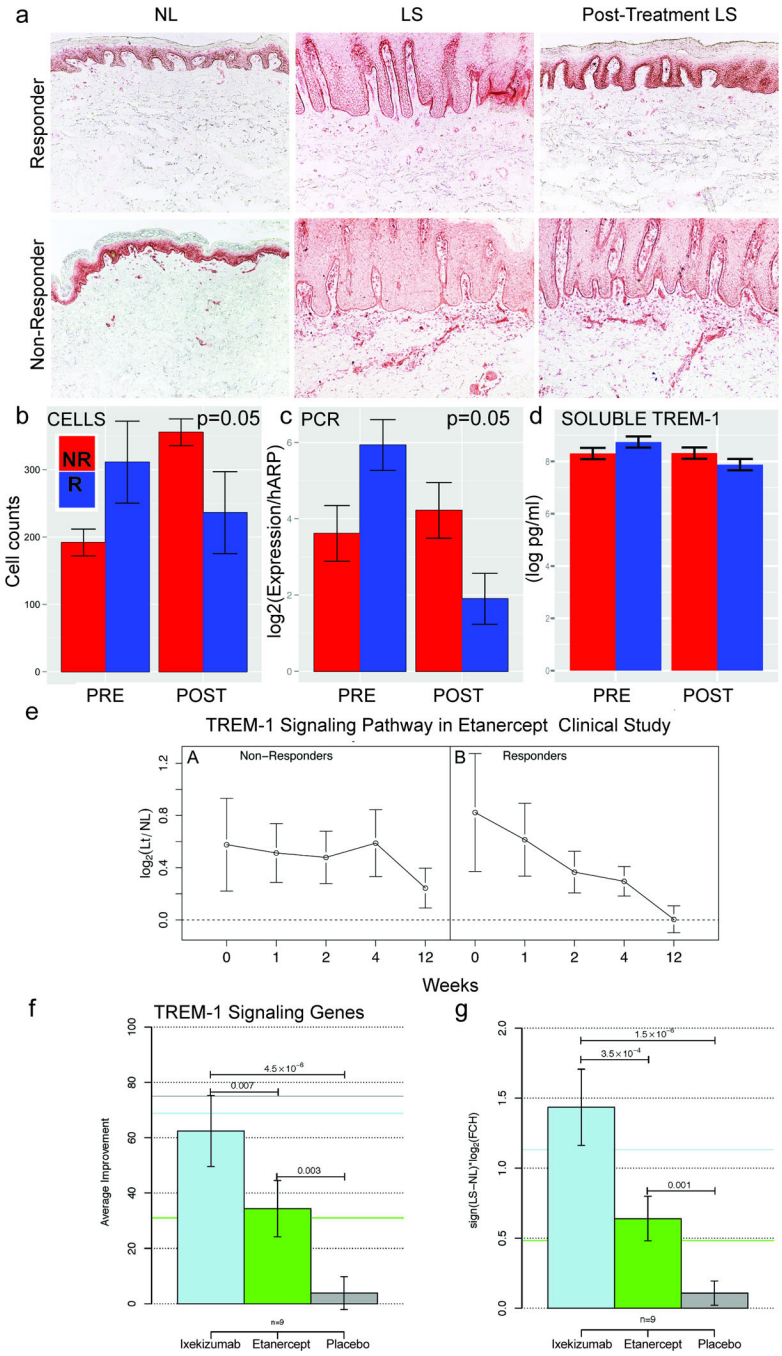

Figure 3. TREM-1 expression was reduced with effective treatment

(a) Representative TREM-1 protein expression at baseline in non-lesional (NL), lesional (LS) and at 6 weeks post-NB-UVB treatment in a responder (upper) and non-responder (lower). (b) TREM-1^{+} cell counts and (c) TREM-1 mRNA expression in the two groups, with a significant effect of treatment on TREM-1 in non-responders (red) and responders (blue) (p=0.05 for both cell counts and mRNA). (d) Soluble TREM-1 in serum of psoriasis patients before and after NB-UVB treatment, grouped as responders and non-responders by *PASI75* (n=7) (Table S4). (e) TREM-1 pathway genes in a group of responders and non-

responders to etanercept treatment (baseline is NL values). (f) Proportion of genes in the TREM-1 pathway that were differentially regulated in psoriasis (n=9) that responded to treatment with IL-17 blockade (Ixekizumab, blue) or TNF blockade (etanercept, green) and placebo (gray) at 2 weeks. Colored lines are changes in all psoriasis genes in the two studies. (g) Mean expression (log_2FCH) of TREM-1 genes towards recovery with ixekizumab, etanercept, or placebo at 2 weeks.

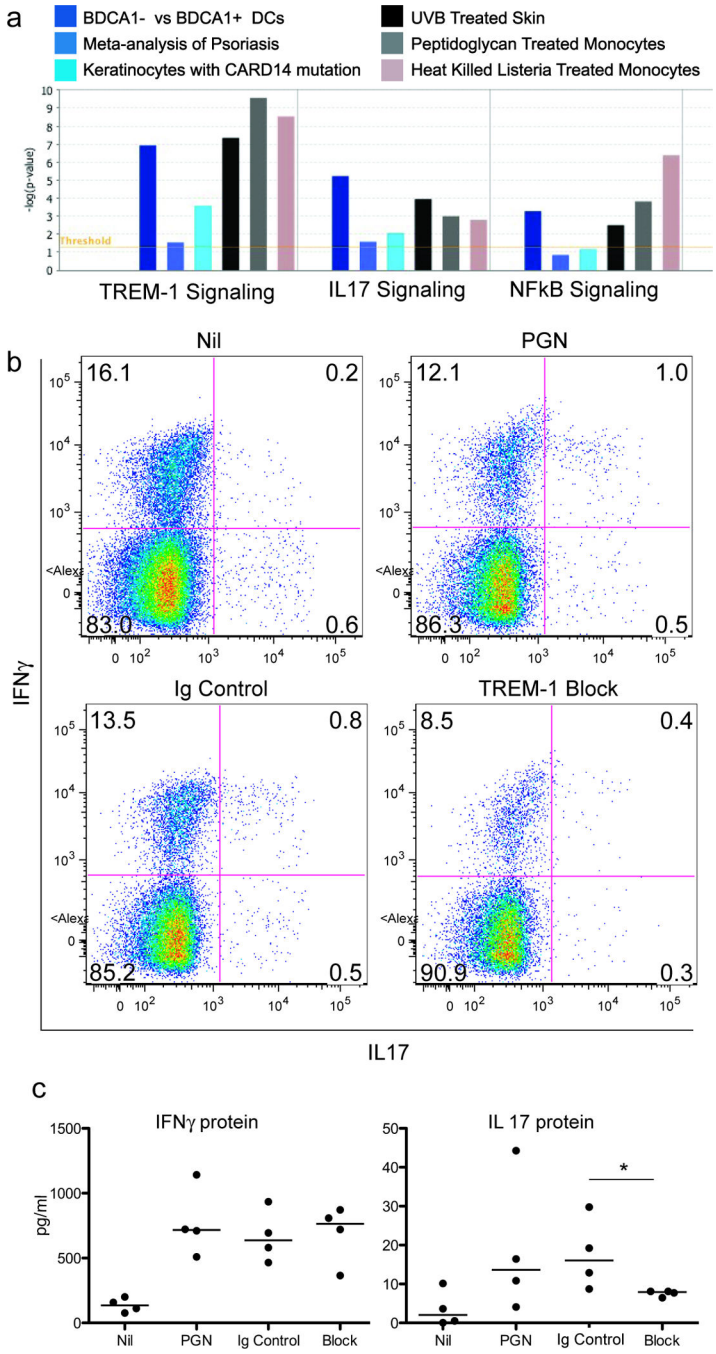

Figure 4. TREM-1 blockade reduced Th17 cells in an *in vitro* allo-MLR

TREM-1, IL-17 and NFκB Signaling canonical pathways for the transcriptomes of psoriatic inflammatory DCs (BDCA-1^{-} versus BDCA-1^{+} DC) (Zaba *et al.*, 2010); psoriasis meta-analysis-derived (MAD) transcriptome (Tian *et al.*, 2012); mutant CARD14 transfected keratinocytes (Jordan *et al.*, 2012a; Jordan *et al.*, 2012b), and UVB-irradiated skin (Kennedy-Crispin *et al.*, 2012); peptidoglycan- and *Heat Killed Listeria Monocytogenes*-treated monocytes compared to untreated monocytes. (b) T cell phenotype for allogeneic T cells after culture (1:10) with monocytes (nil), PGN-treated monocytes (PGN), PGN-treated

monocytes with control antibody (Ig control), and TREM-1 chimera (TREM-1 Block), showing percent IFNγ and IL-17 producing cells by intracellular cytokine staining. Representative of n=2. (c) IFNγ or IL-17 in supernatant of the alloMLR at the time of harvesting day 7 (n=4). *$p<0.05$.

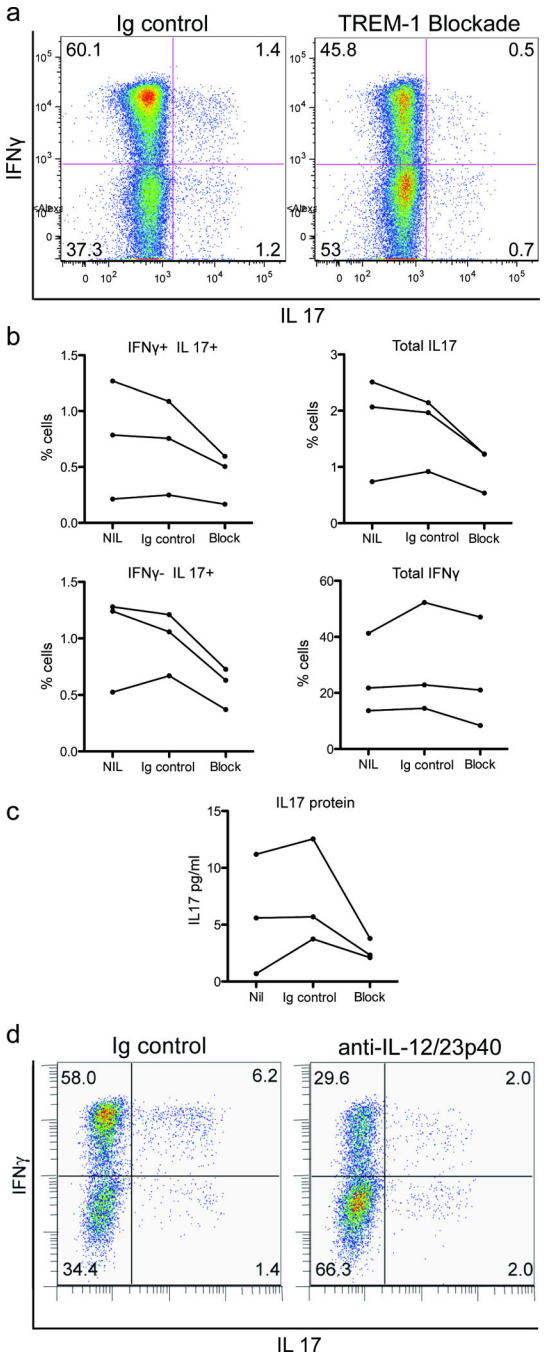

Figure 5. TREM-1 blockade decreases Th17 cells ex vivo

(a) Representative T cell phenotype after allogeneic T cells cultured with psoriatic dermal single cell suspensions with Ig control or TREM-1 blockade showing percent IFNγ and IL-17 producing cells by intracellular cytokine staining. (b) Percentage of T cells producing IL-17 and/or IFNγ (n=3). (c) IL-17 in the supernatant of these experiments. (d) Representative alloMLR with psoriatic dermal single cell suspensions with IgG control or anti-IL-12/23p40 (ustekinumab, Stelara, Janssen) (n=3).